JOURNAL OF VIROLOGY, Apr. 2004, p. 3805–3810 Vol. 78, No. 8
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.8.3805–3810.2004

Transactivator Protein BICP0 of Bovine Herpesvirus 1 (BHV-1) Is Blocked by Prostaglandin D_2 (PGD_2), Which Points to a Mechanism for PGD_2-Mediated Inhibition of BHV-1 Replication

Okay Saydam, Carlos Abril, Bernd Vogt, Mathias Ackermann, and Martin Schwyzer*

Faculty of Veterinary Medicine, Institute of Virology, University of Zurich, CH-8057 Zurich, Switzerland

Received 22 May 2003/Accepted 17 December 2003

The immediate-early protein, BICP0, of bovine herpesvirus 1 (BHV-1) transactivates a variety of viral and cellular genes. In a yeast two-hybrid cDNA library screening, we found that lipocalin-type prostaglandin D synthase, which catalyzes the production of prostaglandin D_2 (PGD_2), is a cellular target of BICP0. We observed that, during wild-type BHV-1 infection, PGD_2 levels were increased intracellularly and decreased in the medium. These effects were absent upon infection with recombinant BHV-1 expressing β-galactosidase instead of BICP0 (A2G2). Transient-expression assays showed that BICP0 alone caused a significant increase in PGD_2 levels in the cell. PGD_2 repressed BHV-1 replication in cultured cells. Antiviral activities of prostaglandins have been documented long ago, but their mode of action remains to be clarified. Here we provide evidence that PGD_2 impairs the transactivation ability of BICP0 that is necessary for efficient virus replication.

The genes of bovine herpesvirus 1 (BHV-1) are expressed in a cascade of three temporally distinct and functionally interdependent phases called immediate-early (IE), early, and late. Three major IE proteins—BHV-1-infected cell protein 0 (BICP0), BICP4, and BICP22—regulate these phases by either transactivating or transrepressing specific virus promoters (11, 27, 48, 55). BICP0 is a key element of BHV-1 replication because of its promiscuous transactivation properties and is considered to be a functional homologue of herpes simplex virus type 1 (HSV-1) ICP0, despite having only limited sequence similarity. The only well-defined domain that is conserved is a C3HC4 zinc ring finger located near the N terminus of both proteins (8, 9, 12). For HSV-1 ICP0, several proteins have been identified as host cell targets: translational elongation factor 1 (24), cyclin D3 (25), and ubiquitin-specific protease (10, 29).

BICP0 has recently been shown to associate with histone deacetylase 1 (57). Since it is well known that IE proteins of alphaherpesviruses are multifunctional, other host cell targets remained to be established. Therefore, we performed a cDNA library screening, from which lipocalin-type prostaglandin D synthase (L-PGDS; EC5.3.99.2; GenBank accession number HUMPROSYN M61901) emerged as one of the host cell targets of BICP0. The enzyme was originally discovered as a major protein of cerebrospinal fluid. It is also localized in several tissues such as brain, heart, eye, testis, and prostate gland. It converts prostaglandin H_2 to prostaglandin D_2 (PGD_2) in the arachidonic acid cascade (50, 53). PGD_2 is the major prostanoid produced in the central nervous system of mammals (32, 35) and has been shown to be involved in the regulation of various physiological responses such as sleep-wake cycle (17, 51), body temperature, hormone release, and pain response (16, 18).

Prostaglandins (PGs) are a class of naturally occurring cyclic 20 carbon fatty acids with potent biological properties. In eukaryotic cells, they are synthesized from polyunsaturated fatty acid precursors derived from the phospholipid pool of the cell membrane in response to external stimuli, such as cell injury and inflammation (5, 13, 54). Prostaglandins are also involved in the control of virus replication and affect the replication of a variety of RNA and DNA viruses, including paramyxoviruses, rhabdoviruses, rotaviruses, retroviruses, and herpesviruses, in cultured cells (42).

In the studies reported here, the major transactivator of BHV-1, BICP0, was found to be blocked by PGD_2, suggesting a potential mechanism for PGD_2-mediated inhibition of BHV-1 replication.

MATERIALS AND METHODS

Yeast two-hybrid screening of cDNA library. A custom MATCHMAKER cDNA library derived from MDBK cells was cloned into plasmid pGAD10 by using the EcoRI cloning site in an adaptor sequence EcoRI-NotI-SalI. The average insert length is 1.8 kbp (Clontech). The parent of the bait plasmid, pBTM116, carries a lexA DNA-binding domain (DBD) coding sequence and a Trp1 gene as a selection marker for yeast (kindly provided by Igor Stagljar, University of Zurich, Zurich, Switzerland). For cDNA library screening of BICP0, we created a bait plasmid designated pBTM116N-BICP0. The EcoRI/SalI fragment (1,096 bp) of pBORF26 (12) was inserted into pBTM116 cut with EcoRI and SalI. The resulting plasmid pBTM116N-BICP0 expresses a hybrid protein consisting of the LexA DBD fused to the N-terminal half part of BICP0 (amino acids [aa] 1 to 357). The control plasmid, pBTM116LaminC, encoding a noncognate protein used in the bait dependency test was created as follows. The EcoRI/PstI fragment (513 bp) of pLAM5′ (Clontech, Palo Alto, Calif.) was inserted into pBTM116 cut with EcoRI and PstI. The resulting plasmid, pBTM116LaminC, encodes a fusion protein consisting of the LexA DBD fused to human lamin C protein (aa 66 to 230). Yeast transformations (15) and DNA preparation from yeast (22) were carried out as described previously. Positive clones were identified by X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) filter assay, measuring β-galactosidase activity as described previously (47). One of the cDNA library clones emerging from the screening and examined in this report was pGAD10L-PGDS which encodes L-PGDS (aa 50 to 190).

* Corresponding author. Mailing address: Institute of Virology, Faculty of Veterinary Medicine, University of Zurich, Winterthurerstrasse 266A, CH-8057 Zurich, Switzerland. Phone: 41-1-6358704. Fax: 41-1-6358911. E-mail: schwyzer@vetvir.unizh.ch.

Cell culture. HeLa cells were cultured in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 100 U of penicillin/ml, and 100 μg of streptomycin/ml in 5% CO_2 at 37°C. MDBK cells were cultured in Eagle minimal essential medium (EMEM) supplemented with 10% fetal bovine serum, 100 U of penicillin/ml, and 100 μg of streptomycin/ml at 37°C.

Virus purification. Virus was purified (30) from MDBK cells infected with wild-type BHV-1 strain Jura or with A2G2, a recombinant form of BHV-1 in which the BICP0 gene is disrupted by a β-galactosidase expression cassette (26). When the cytopathic effect was 90% complete, the bulk of the medium was removed; cells were frozen and thawed three times, sonicated in an MSE sonifier for 20 s with 20% output energy, and then mixed again with the medium and centrifuged at 12,000 × *g* for 5 min to remove cell debris. From the supernatant, virus was pelleted by centrifugation in an SW28 rotor for 2 h at 120,000 × *g* through a cushion of 15 ml of 30% sucrose. The pellet was resuspended in 0.5 ml of TNE (150 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA [pH 7.5]), layered onto a linear 20 to 60% sucrose gradient in TNE and centrifuged at 120,000 × *g* in an SW28 rotor. The visible virus-containing band was aspirated with a syringe, and virus was pelleted and resuspended in TNE as described above.

Virus infection. A total of 3×10^5 MDBK cells were seeded in each well of a six-well plate and infected on the next day with BHV-1 strain Jura (multiplicity of infection [MOI] = 3) in 1 ml of EMEM without fetal calf serum (FCS). After 2 h of adsorption at 37°C, the inoculum was removed. Cell monolayers were fed with 1 ml of EMEM containing 2% FCS and the indicated concentrations of PGD_2. After a 9-h exposure to PGD_2, cells were harvested together with the medium. From 200 μl of this mixture, viral DNA was isolated by using the QIAamp DNA Mini kit (Qiagen) according to the manufacturer's blood and body fluid spin protocol and then analyzed by real-time PCR (TaqMan; Applied Biosystems).

Fluorogenic PCR for the detection and quantification of BHV-1 DNA. The glycoprotein gB gene was targeted for amplification of 97 nucleotides from the viral DNA by the TaqMan technique. The experimental conditions were as described previously (23), with the following exceptions. The sequences of the oligonucleotides used were as follows: 5′-TGT GGA CCT AAA CCT CAC GGT-3′ (forward primer), 5′-GTA GTC GAG CAG ACC CGT GTC-3′ (reverse primer), and 5′-FAM-AGG ACC GCG AGT TCT TGC CGC-TAMRA-3′ (probe). PCR amplifications were carried out in a volume of 25 μl, containing 12.5 μl of Mastermix (PE Applied Biosystems), 2.5 μl of each primer (240 nM), probe (160 nM), and diethyl pyrocarbonate-treated water, and 10 μl of DNA sample. After 2 min at 50°C and 10 min at 95°C, 40 cycles were carried out with denaturation at 95°C for 15 s and annealing-elongation at 60°C for 1 min. Standard curves were generated from serial dilutions of plasmid cloned viral DNA. In each single assay 10 copies (Ct value of 38.23 ± 1.89) or more of the template were detected. The Ct values decreased with increasing template concentration in a linear fashion over a range of 10 orders of magnitude. A template concentration of 10^{10} molecules per assay correlated to a Ct value of 8.77. Similarly, a correlation between infectious units ($TCID_{50}$) and amplification cycles (Ct value) was established by using viral DNA extracted from infected cell cultures as a template. A correlate to 1.5 $TCID_{50}$ of BHV-1 was detected in each single assay, and the standard curve remained linear up to a virus concentration of $10^{8.2}$ $TCID_{50}$/ml.

PGD_2 measurement. MDBK cells were infected with purified wild-type BHV-1 or A2G2 (MOI = 1) or mock infected. Media were collected and cells were harvested at 3 and 6 h of postinfection. Alternatively, HeLa cells were transfected with a BICP0-expressing plasmid, pBCMV26, or a pBS KS+ control plasmid. A transfection efficiency of 30 to 40% was reached. Media were collected and cells were harvested 40 h after transfection. Cells were lysed by sonification. Protein concentration of the cell lysates was determined by a Bradford assay (Bio-Rad). After solvent extraction and chromatography on Amprep RNP1903 (Amersham Pharmacia) according to the manufacturer's instructions, the PGD_2 levels in media and cell extracts were measured by using a PGD_2 radioimmunoassay kit containing ^{3}H-labeled PGD_2 and a polyclonal rabbit antiserum against conjugated PGD_2 (Hartmann Analytic). The principle of the test is that the radioactive tracer competes with nonradioactive PGD_2 in samples for the limited number of antibody-binding sites.

Plasmids for transient-expression assays. The effector plasmid, pBCMV26, contains the BICP0 coding sequence under the control of the human cytomegalovirus (CMV) IE promoter (27). The reporter plasmid, pC29CAT, contains the chloramphenicol acetyltransferase (CAT) gene under control of the IE4.2/2.9 promoter (55). The reporter plasmid, pG13-luc, contains a luciferase reporter gene under control of a tandem repeat of thirteen p53 binding sequences without any other *cis*-acting elements (37).

Exposure of transfected cells to PGD_2. PGD_2 was purchased from Calbiochem and dissolved in absolute ethanol to prepare 3 mM stock solution. HeLa cells were transfected by the calcium phosphate method. Cells were treated with various amounts of PGD_2 36 h after transfection for 4 h. Cells were then harvested and subjected to CAT or luciferase assays.

Cytotoxicity. Cytotoxicity in the cell culture was determined after treating triplicate confluent cultures of HeLa cells with various amounts of PGD_2. Cultures were observed by phase-contrast microscopy several times after PGD_2 treatment. After 4 h exposure to PGD_2, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to the cultures to achieve a final concentration of 500 μg/ml. Cultures were incubated for an additional 2 h, medium was removed, and dimethyl sulfoxide (150 μl/well) was added to dissolve the MTT-formazan product. At 250 μM PGD_2, the amount of the MTT-formazan product, measured by absorbance at 540 nm, was reduced by 17% relative to the untreated control. At 30 μM PGD_2, the highest concentration used in the present study, no cytotoxicity could be detected.

CAT assay. HeLa cells were plated with a density of 4×10^5 per well in six-well plates 24 h before transfection. The effector plasmid pBCMV26 (2 μg) and reporter plasmid pC29CAT (1 μg) were cotransfected by the calcium phosphate method. A CAT assay was performed as described elsewhere in detail (55). Briefly, cells were harvested in phosphate-buffered saline 40 h after transfection and lysed by three cycles of freezing and thawing. CAT activity was measured. Chloramphenicol and its acetylated forms were separated by thin-layer chromatography. The amounts of acetylated chloramphenicol were measured with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

Luciferase assay. A total of 1 μg of pBCMV26 and 1 μg of pG13-luc was used for transient expression assays in six-well plates. HeLa cells were harvested 40 h after transfection and lysed by three cycles of freezing and thawing. After centrifugation (14,000 rpm, 5 min), 10 μl of supernatant was mixed with 100 μl of luciferase assay solution (Promega, Madison, Wis.), and the mixture was analyzed in a luminometer.

Statistical analysis. All values were expressed as mean ± the standard error of the mean; *P* values were calculated by using unpaired *t* test, and values of $P < 0.05$ were regarded as significant.

TABLE 1. BICP0 interacts with L-PGDS in yeast as determined by an X-Gal filter assay for galactosidase activity

Plasmid combination	Description	X-Gal filter
pBTM116N-BICP0 + pGAD10L-PGDS	Target	Blue
pBTM116Lamin C + pGAD10L-PGDS	Negative control	White
pBTM116LexA + pGAD10L-PGDS	Negative control	White

RESULTS

L-PGDS is a host cell target of BICP0. To search for potential host cell targets of the IE protein, BICP0, we performed a cDNA library screening by using the yeast two-hybrid system. Several bait-dependent clones were isolated and characterized by sequencing and database searching; these will be reported elsewhere. One of the clones, pGAD10L-PGDS, was found to encode lipocalin-type prostaglandin D synthase (L-PGDS) and led to the studies presented here.

To test whether the observed L-PGDS–BICP0 interaction is specific for BICP0, we performed additional yeast transformations, including as a control human lamin C protein, which has been reported not to form complexes nor to interact with most other proteins (2, 56). The yeast strain L40 was cotransformed with pGAD10L-PGDS and either pBTM116N-BICP0 encoding the N-terminal half of BICP0, pBTM116LaminC encoding human lamin C protein, or empty vector (pBTM116). After colonies appeared, X-Gal filter assay was performed. Only yeasts cotransformed with pGAD10L-PGDS and pBTM116N-BICP0 exhibited blue staining, whereas neither lamin C nor LexA (empty vector) interacted with L-PGDS (Table 1). Co-

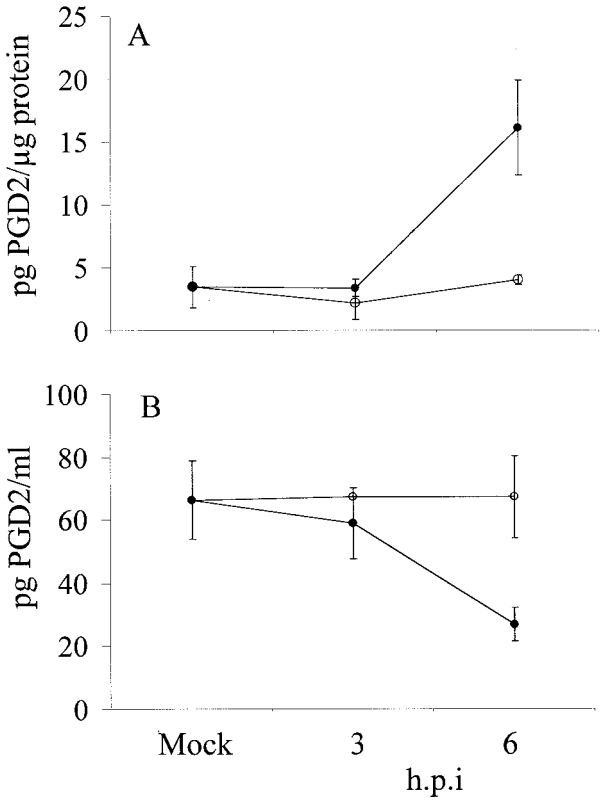

FIG. 1. Effect of BHV-1 infection on PGD_2 levels. MDBK cells were infected at an MOI of 1 with purified wild-type BHV-1 (●) or recombinant BHV-1 A2G2 (○) or were mock infected. At the indicated times after the end of the adsorption period (hours postinoculation), media were collected and cells were harvested. PGD_2 levels in cell extracts (A) and in the media (B) were measured by radioimmunoassay.

immunoprecipitation assays (not shown) also confirmed the specificity of the interaction.

Infection with BHV-1 raises intracellular PGD_2 levels. Since L-PGDS is an enzyme that is responsible for the formation of PGD_2, we first measured by radioimmunoassay the PGD_2 levels during wild-type BHV-1 infection. During the first 3 h of infection, we observed no change in cell-associated PGD_2 levels, but during the next 3 h, when IE proteins were expected to have full effect, the PGD_2 levels rose fivefold (Fig. 1A). This increase could be attributed to the presence of BICP0 because A2G2, a recombinant BHV-1 in which the BICP0 gene is disrupted by a β-galactosidase expression cassette, did not significantly affect the PGD_2 levels.

Upon production, prostaglandins are released rapidly from cells and act near their site of production by binding to specific cellular receptors (3, 6, 33). Therefore, we also measured PGD_2 levels in the medium. As shown in Fig. 1B, the PGD_2 level was found to be decreased at 6 h of infection compared to the media of mock-infected cells. No significant decrease was observed at 3 h of infection. This decrease could also be attributed to the presence of BICP0 because infection with A2G2 had no such effect.

Transient expression of BICP0 is sufficient to raise intracellular PGD_2 levels. Next, we examined whether BICP0 might exhibit a similar effect on the PGD_2 production in the absence of viral infection. HeLa cells were transfected with a BICP0-expressing plasmid, pBCMV26. After 40 h of transfection, media were collected and cells were harvested. PGD_2 levels were measured both in the whole-cell extract and in the media. As shown in Fig. 2, transient BICP0 expression led to a significant increase in PGD_2 levels in the cells ($P < 0.05$), whereas the reduction in PGD_2 levels in the media of the transfected cells was not considered significant ($P > 0.05$).

PGD_2 inhibits BHV-1 replication. Prostaglandins have been shown to activate (1, 31) or repress (38, 39, 42–46) the replication of various viruses. To test whether BHV-1 replication is affected by PGD_2, we infected MDBK cells with wt BHV-1. After a 2-h adsorption period, different concentrations of PGD_2 were added to the culture, and the accumulation of viral DNA was determined by TaqMan PCR for the gB gene of BHV-1 at 9 h postinfection. We found that PGD_2 reduced BHV-1 replication in a dose-dependent manner and inhibited the virus yield by 300-fold at the highest concentration of 100 nM (Fig. 3A). The solvent alone (ethanol) did not affect virus replication at the concentrations used (not shown).

To determine the effect of PGD_2 on viral protein levels, virus infection and PGD_2 treatment were performed as described above. Cells were harvested at 9 h postinfection, and cell extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. To detect viral proteins, we performed a Western blot with serum obtained from a BHV-1-infected cow. As shown in Fig. 3B, we found that PGD_2 treatment caused a marked reduction in the viral pro-

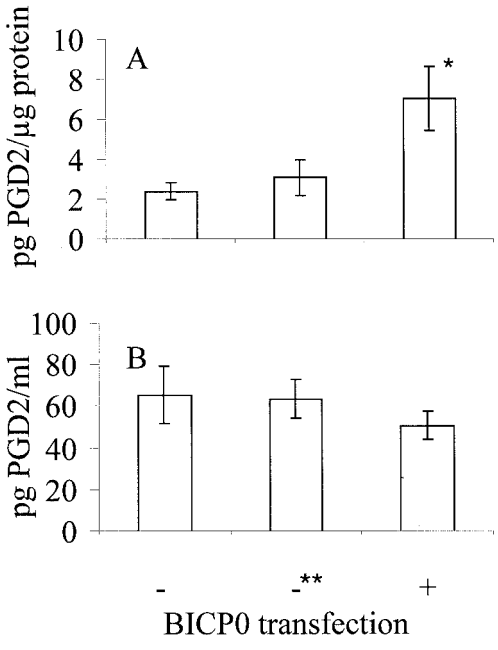

FIG. 2. PGD_2 levels after transient BICP0 expression. HeLa cells were transfected with pBCMV26 (right), with control plasmid pBS KS+ (middle), or left without transfection (left). Media were collected and cells were harvested 40 h after transfection. PGD_2 levels were measured by radioimmunoassay in cell extracts (A) and in the media (B). ✻, Significant increase in PGD_2 levels ($P < 0.05$); ✻✻, control plasmid.

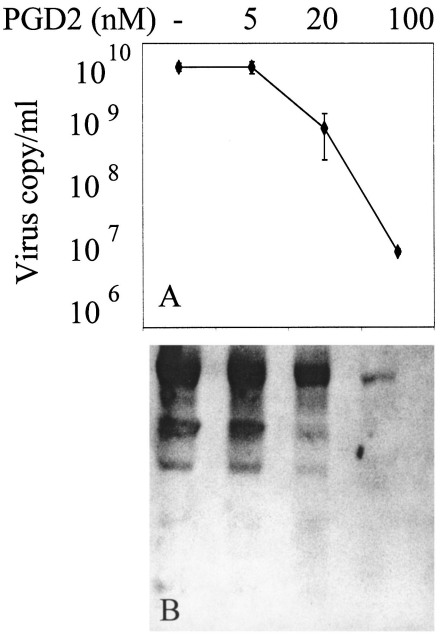

FIG. 3. Effect of PGD_2 on BHV-1 replication. (A) PGD_2 inhibits BHV-1 replication. Confluent monolayers of MDBK cells were infected with wild-type BHV-1 with an MOI of 3. After a 2-h adsorption period, the medium was replaced by 1 ml of EMEM supplemented with 2% fetal bovine serum and various concentrations of PGD_2. After 9 h of infection, both cell-associated and free viral DNAs were detected with TaqMan PCR as described in Materials and Methods. (B) Viral proteins are reduced by PGD_2. BHV-1 infection and PGD_2 treatment were performed as described above. Cells were harvested 9 h after infection and Western blot analysis was performed with an anti-BHV-1 serum obtained from a BHV-1-infected cow.

tein amount at concentration 20 and 100 nM. There was no significant reduction at the concentration of 5 nM.

PGD_2 inhibits BICP0-dependent transactivation. The major BHV-1 IE protein and transactivator BICP0 is known to be required for efficient BHV-1 replication. Therefore, we examined the possibility that BICP0 was a target of PGD_2-mediated inhibitory action. HeLa cells were cotransfected with pBCMV26, a plasmid expressing BICP0, and pC29CAT, in which the CAT gene is expressed under a BICP0-dependent viral promoter. At 36 h after transfection, cells were treated with different concentrations of PGD_2 for 4 h. At the end of the treatment period, cells were harvested and CAT activity was measured. As shown in Fig. 4A, BICP0 activated that promoter as expected. BICP0-dependent promoter activity was reduced by PGD_2 in a dose-dependent manner. In contrast, the basal promoter activity of pC29CAT in the absence of stimulatory BICP0 was not inhibited by PGD_2 (Fig. 4A). To determine whether the effect of PGD_2 depends on a specific viral promoter, we next examined a nonviral reporter construct, pG13-luc that contains a luciferase gene under control of 13 consensus binding sites for p53 protein. That promoter was found to be significantly activated by BICP0. Similarly to the results above, BICP0-dependent promoter activity was reduced by PGD_2 in a dose-dependent manner, whereas the basal promoter activity was not affected (Fig. 4B). We concluded that PGD_2 may specifically block BICP0-mediated transactivation at concentrations considerably less than those required to produce measurable cytotoxicity by MTT assay (see Materials and Methods).

Effect of PGD_2 treatment on the BICP0 protein. The prostaglandins PGA_1 and PGJ_2 have been shown to inhibit the glycosylation of the vesicular stomatitis virus glycoprotein G, altering its electrophoretic mobility and decreasing its molecular mass by 4 kDa (31, 38, 46). PGJ_2 has also been shown to selectively inhibit the glycosylation, maturation, and intracellular translocation of the Sendai virus glycoprotein HN (45). To test whether PGD_2 can affect protein maturation or posttranslational modification of BICP0, transient-expression assay and PGD_2 treatment were performed as described above. Cell extracts were prepared and analyzed by Western blot probed with anti-BICP0 antibody. Neither the protein amount nor the electrophoretic mobility of BICP0 protein was affected by PGD_2. The same membrane was later reprobed with anti-actin antibody as a control for loading of the gel (Fig. 5).

DISCUSSION

Prostaglandins function as microenvironmental hormones involved in the regulation of physiological and pathological processes, including inflammation, cytoprotection, and virus infection (18, 42, 51). In the present study, we showed that BHV-1 increases PGD_2 levels in the cells at 6 h postinfection

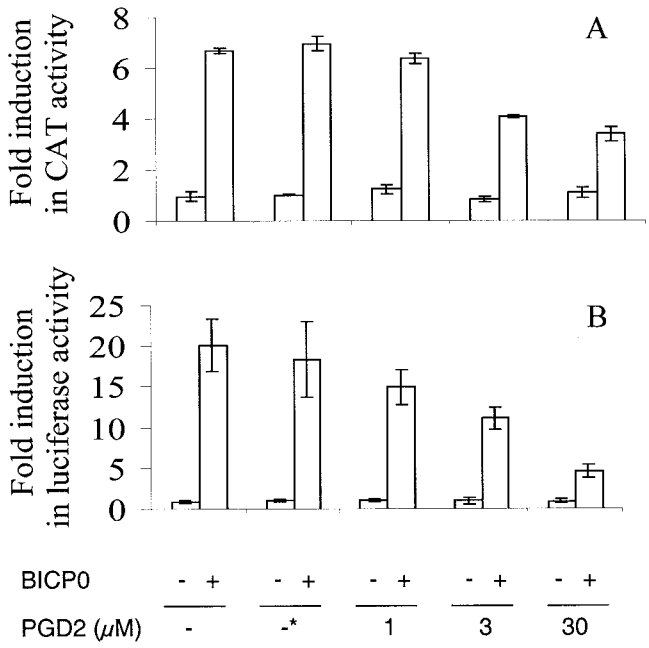

FIG. 4. PGD_2 inhibits BICP0-dependent gene regulation. HeLa cells were cotransfected either with pC29CAT and pBCMV26 or pG13-luc and pBCMV26. At 36 h after transfection, cells were treated with PGD_2 for 4 h and then harvested. CAT and luciferase assays were performed as described in Materials and Methods. The data presented are the mean values with the standard deviations from three independent experiments. The basal level in the absence of pBCMV26 and PGD_2 was taken as 1 in order to calculate fold activation. (A) The effect of PGD_2 on the reporter plasmid pC29CAT; (B) the effect of PGD_2 on the nonviral promoter, pG13-luc, activated by BICP0. ✻, Solvent control.

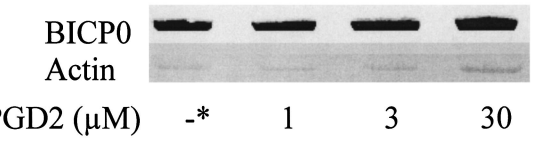

FIG. 5. Effect of PGD_2 treatment on the BICP0 protein. Transfection and PGD_2 treatment were performed as carried out for Fig. 4. At the end of treatment, cells were harvested. Proteins were separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. Immunoblot was first performed with anti-BICP0 antibody. Then after an extensive wash and overnight blocking, the same membrane was incubated with anti-actin antibody. *, Solvent control.

and decreases them in the media. This observation was first made by using unpurified virus lysates (not shown). Considering the possibility that virus infection might induce cellular factors that could account for these effects, we then purified the virus particles by several centrifugation steps. The result shown in Fig. 1 with purified virus was quantitatively rather similar to that with unpurified virus. Furthermore, A2G2, a recombinant BHV-1 in which the BICP0 gene is disrupted by a β-galactosidase expression cassette, did not exert these effects, suggesting that BICP0 was the agent causing these changes in PGD_2 levels.

Next, we undertook a series of experiments to further examine the possible role of PGD_2 in BHV-1 infection. We found that PGD_2 inhibits BHV-1 replication. The virus yield was reduced 300-fold at the highest PGD_2 concentration used (Fig. 3A). The inhibitory action of PGD_2 on virus growth was correlated with a reduced amount of viral proteins (Fig. 3B). These events could be considered as a host cell defense mechanism. The concentrations of exogenously added PGD_2 in the present study are significantly higher than concentrations measured in BHV-1-infected cells. Prostaglandins are physiologically present in body fluids at picomolar-to-nanomolar concentrations (49); however, arachidonic-acid metabolism is highly increased in several pathological conditions, including hyperthermia, infection, and inflammation (4, 19, 28), and local prostaglandin concentrations in the micromolar range have been detected at sites of acute inflammation (34) and in seminal fluid (14, 40). Therefore, concentrations of PGD_2 that are sufficient to inhibit BHV-1 replication appear to be physiologically relevant.

Antiviral activities of prostaglandins have been documented long ago, but their mode of action remains to be clarified. Here we provide evidence that PGD_2 impairs the transactivation ability of BICP0 that is necessary for efficient virus replication (Fig. 4). Based on the observation that the viral proteins are reduced by PGD_2 during BHV-1 infection (Fig. 3B), the possibility had to be considered that PGD_2 would alter the levels of BICP0 in transient expression assay or influence its post-translational modification or intracellular localization. However, the Western blot shown in Fig. 5 revealed that PGD_2 did not change the levels of BICP0 protein. The apparent molecular mass of BICP0 protein, 97 kDa, was not affected by PGD_2 either, suggesting that PGD_2 has no major effect on the post-translational modification of the BICP0 protein. This does not exclude the possibility of minor differences, for example, in phosphorylation patterns. To determine whether PGD_2 affects the intracellular localization of BICP0, a similar experiment to that shown in Fig. 4 was performed with a BICP0-green fluorescent protein fusion protein expression plasmid. Fluorescence microscopy revealed that the nuclear localization of BICP0 remained unchanged (not shown).

Cyclopentenone prostaglandins are characterized by the presence of a ring system containing an electrophilic carbon. This ring system can react covalently by means of the Michael addition reaction with nucleophiles such as free sulfhydryls of glutathione and cysteine residues in cellular proteins (42). PGJ_2 and PGA have been shown to inhibit NF-κB and p53-dependent gene expression through covalent modification of critical cysteine residues in IκB kinase and the DBDs of NF-κB subunits or conformational change in the p53 protein, respectively (7, 36, 41). Although, from these data, it seems possible that the activity of BICP0 could be modulated in a similar manner to NF-κB and p53, the present study should stimulate further investigation to find out the mechanism(s) by which PGD_2 blocks BICP0-dependent transactivation. Taken together, our results indicate that block of BICP0 can play an important role in the PGD_2 induced-inhibition of BHV-1 replication. However, since it is known that prostaglandins can act at multiple levels during the virus replication cycle (42), we do not exclude that other mechanisms could contribute to the inhibition of BHV-1 replication by PGD_2.

Recent studies have shown that PGE_2 receptor EP4 (20) and cyclooxygenase 2 (21) genes are upregulated during HSV-1 reactivation triggered by cyclophosphamide and dexamethasone or heat shock. It is likely that BICP0 carries out its role in activation of transcription and reactivation from latency by interacting with cellular factors. One of the factors interacting with BICP0 is L-PGDS; it is known as the brain-type enzyme, predominantly expressed in neurons and oligodendrocytes (50, 52). PGD_2 is the main archidonic acid metabolite produced by L-PGDS in neurons and glial cells in the mammalian central nervous system (51). Based on these connections, we postulate that BICP0 may target L-PGDS and PGD_2 synthesis either by inhibiting the enzyme directly, by sequestering it, or by marking it for proteasome degradation. Furthermore, we consider that the triad of BICP0, L-PGDS, and PGD_2 may have a role in shifting the balance between latent and lytic BHV-1 infection.

ACKNOWLEDGMENTS

We thank Adrian Fairburn for help and advice during the PGD_2 measurement and Cornel Fraefel, Hanspeter Hefti, and Kurt Tobler for help and discussions.

This study was supported by grants 3100-053751.98 and 3100-065396.01 from the Swiss National Science Foundation.

REFERENCES

1. **Baker, D. A., J. Thomas, J. Epstein, D. Possilico, and M. L. Stone.** 1982. The effect of prostaglandins on the multiplication and cell-to-cell spread of herpes simplex virus type 2 in vitro. Am. J. Obstet. Gynecol. **144:**346–349.
2. **Bartel, P., C. T. Chien, R. Sternglanz, and S. Fields.** 1993. Elimination of false positives that arise in using the two-hybrid system. BioTechniques **14:**920–924.
3. **Breyer, R. M., C. K. Bagdassarian, S. A. Myers, and M. D. Breyer.** 2001. Prostanoid receptors: subtypes and signaling. Annu. Rev. Pharmacol. Toxicol. **41:**661–690.
4. **Calderwood, S. K., B. Bornstein, E. K. Farnum, and M. A. Stevenson.** 1989. Heat shock stimulates the release of arachidonic acid and the synthesis of prostaglandins and leukotriene B4 in mammalian cells. J. Cell. Physiol. **141:**325–333.

5. **Coffman, T. M., B. H. Koller, and S. L. Tilley.** 2001. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. J. Clin. Investig. **108:**15–23.
6. **Dussault, I., and B. M. Forman.** 2000. Prostaglandins and fatty acids regulate transcriptional signaling via the peroxisome proliferator activated receptor nuclear receptors. Prostaglandins Other Lipid Mediat. **62:**1–13.
7. **Edes, K., F. A. Fitzpatrick, and P. J. Moos.** 2000. Inactivation of wild-type p53 tumor suppressor by electrophilic prostaglandins. Proc. Natl. Acad. Sci. USA **97:**9215–9220.
8. **Everett, R. D.** 1988. Analysis of the functional domains of herpes simplex virus type 1 immediate-early polypeptide Vmw110. J. Mol. Biol. **202:**87–96.
9. **Everett, R. D.** 2000. ICP0, a regulator of herpes simplex virus during lytic and latent infection. Bioessays 2**2:**761–770.
10. **Everett, R. D., M. Meredith, A. Orr, A. Cross, M. Kathoria, and J. Parkinson.** 1997. A novel ubiquitin-specific protease is dynamically associated with the PML nuclear domain and binds to a herpesvirus regulatory protein. EMBO J. **16:**1519–1530.
11. **Fraefel, C., M. Ackermann, and M. Schwyzer.** 1994. Identification of the bovine herpesvirus 1 Circ protein, a myristylated and virion-associated polypeptide which is not essential for virus replication in cell culture. J. Virol. **68:**8082–8088.
12. **Fraefel, C., J. Zeng, Y. Choffat, M. Engels, M. Schwyzer, and M. Ackermann.** 1994. Identification and zinc dependence of the bovine herpesvirus 1 transactivator protein BICP0. J. Virol. **68:**3154–3162.
13. **Funk, C. D.** 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science **294:**1871–1875.
14. **Gerozissis, K., P. Jouannet, J. C. Soufir, and F. Dray.** 1982. Origin of prostaglandins in human semen. J. Reprod. Fertil. **65:**401–404.
15. **Gietz, R. D., R. H. Schiestl, A. R. Willems, and R. A. Woods.** 1995. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast **11:**355–360.
16. **Hayaishi, O.** 2002. Molecular genetic studies on sleep-wake regulation, with special emphasis on the prostaglandin D_2 system. J. Appl. Physiol. **92:**863–868.
17. **Hayaishi, O.** 2000. Molecular mechanisms of sleep-wake regulation: a role of prostaglandin D2. Philos. Trans. R. Soc. Lond. B Biol. Sci. **355:**275–280.
18. **Hayaishi, O., and Y. Urade.** 2002. Prostaglandin D2 in sleep-wake regulation: recent progress and perspectives. Neuroscientist **8:**12–15.
19. **Herschman, H. R., S. T. Reddy, and W. Xie.** 1997. Function and regulation of prostaglandin synthase-2. Adv. Exp. Med. Biol. **407:**61–66.
20. **Higaki, S., B. M. Gebhardt, W. J. Lukiw, H. W. Thompson, and J. M. Hill.** 2002. Effect of immunosuppression on gene expression in the HSV-1 latently infected mouse trigeminal ganglion. Investig. Ophthalmol. Vis. Sci. **43:**1862–1869.
21. **Hill, J. M., W. J. Lukiw, B. M. Gebhardt, S. Higaki, J. M. Loutsch, M. E. Myles, H. W. Thompson, B. S. Kwon, N. G. Bazan, and H. E. Kaufman.** 2001. Gene expression analyzed by microarrays in HSV-1 latent mouse trigeminal ganglion following heat stress. Virus Genes **23:**273–280.
22. **Hoffman, C. S., and F. Winston.** 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:**267–272.
23. **Hüssy, D., N. Stäuber, C. M. Leutenegger, S. Rieder, and M. Ackermann.** 2001. Quantitative fluorogenic PCR assay for measuring ovine herpesvirus 2 replication in sheep. Clin. Diagn. Lab. Immunol. **8:**123–128.
24. **Kawaguchi, Y., R. Bruni, and B. Roizman.** 1997. Interaction of herpes simplex virus 1 alpha regulatory protein ICP0 with elongation factor 1delta: ICP0 affects translational machinery. J. Virol. **71:**1019–1024.
25. **Kawaguchi, Y., C. Van Sant, and B. Roizman.** 1997. Herpes simplex virus 1 alpha regulatory protein ICP0 interacts with and stabilizes the cell cycle regulator cyclin D3. J. Virol. **71:**7328–7336.
26. **Köppel, R., C. Fraefel, B. Vogt, L. J. Bello, W. C. Lawrence, and M. Schwyzer.** 1996. Recombinant bovine herpesvirus-1 (BHV-1) lacking transactivator protein BICP0 entails lack of glycoprotein C and severely reduced infectivity. Biol. Chem. **377:**787–795.
27. **Köppel, R., B. Vogt, and M. Schwyzer.** 1997. Immediate-early protein BICP22 of bovine herpesvirus 1 trans-represses viral promoters of different kinetic classes and is itself regulated by BICP0 at transcriptional and post-transcriptional levels. Arch. Virol. **142:**2447–2464.
28. **Leung, D. Y., L. Key, J. J. Steinberg, M. C. Young, M. Von Deck, R. Wilkinson, and R. S. Geha.** 1988. Increased in vitro bone resorption by monocytes in the hyper-immunoglobulin E syndrome. J. Immunol. **140:**84–88.
29. **Meredith, M., A. Orr, and R. Everett.** 1994. Herpes simplex virus type 1 immediate-early protein Vmw110 binds strongly and specifically to a 135-kDa cellular protein. Virology **200:**457–469.
30. **Metzler, A. E., A. A. Schudel, and M. Engels.** 1986. Bovine herpesvirus 1: molecular and antigenic characteristics of variant viruses isolated from calves with neurological disease. Arch. Virol. **87:**205–217.
31. **Moriuchi, M., H. Inoue, and H. Moriuchi.** 2001. Reciprocal interactions between human T-lymphotropic virus type 1 and prostaglandins: implications for viral transmission. J. Virol. **75:**192–198.
32. **Narumiya, S., T. Ogorochi, K. Nakao, and O. Hayaishi.** 1982. Prostaglandin D2 in rat brain, spinal cord and pituitary: basal level and regional distribution. Life Sci. **31:**2093–2103.
33. **Narumiya, S., Y. Sugimoto, and F. Ushikubi.** 1999. Prostanoid receptors: structures, properties, and functions. Physiol. Rev. **79:**1193–1226.
34. **Offenbacher, S., B. M. Odle, and T. E. Van Dyke.** 1986. The use of crevicular fluid prostaglandin E_2 levels as a predictor of periodontal attachment loss. J. Periodontal Res. **21:**101–112.
35. **Ogorochi, T., S. Narumiya, N. Mizuno, K. Yamashita, H. Miyazaki, and O. Hayaishi.** 1984. Regional distribution of prostaglandins D2, E2, and F2 alpha and related enzymes in postmortem human brain. J. Neurochem. **43:**71–82.
36. **Pascual, G., M. Li, J. S. Welch, M. Ricote, C. H. Hsiang, L. L. Sengchanthalangsy, G. Ghosh, C. K. Glass, and D. S. Straus.** 2000. 15-Deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-κB signaling pathway. Proc. Natl. Acad. Sci. USA **97:**4844–4849.
37. **Petersen, G. M., A. J. Krush, S. Booker, J. Jen, F. M. Giardiello, S. R. Hamilton, B. Vogelstein, K. W. Kinzler, and W. S. el-Deiry.** 1993. WAF1, a potential mediator of p53 tumor suppression. N. Engl. J. Med. **329:**1982–1987.
38. **Pica, F., A. De Marco, F. De Cesare, and M. G. Santoro.** 1993. Inhibition of vesicular stomatitis virus replication by delta 12-prostaglandin J2 is regulated at two separate levels and is associated with induction of stress protein synthesis. Antivir. Res. **20:**193–208.
39. **Pica, F., A. T. Palamara, A. Rossi, A. De Marco, C. Amici, and M. G. Santoro.** 2000. $Delta_{12}$-prostaglandin J_2 is a potent inhibitor of influenza A virus replication. Antimicrob. Agents Chemother. **44:**200–204.
40. **Quayle, A. J., R. W. Kelly, T. B. Hargreave, and K. James.** 1989. Immunosuppression by seminal prostaglandins. Clin. Exp. Immunol. **75:**387–391.
41. **Rossi, A., P. Kapahi, G. Natoli, T. Takahashi, Y. Chen, M. Karin, and M. G. Santoro.** 2000. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IκB kinase. Nature **403:**103–108.
42. **Santoro, M. G.** 1997. Antiviral activity of cyclopentenone prostanoids. Trends Microbiol. **5:**276–281.
43. **Santoro, M. G., C. Amici, G. Elia, A. Benedetto, and E. Garaci.** 1989. Inhibition of virus protein glycosylation as the mechanism of the antiviral action of prostaglandin A in Sendai virus-infected cells. J. Gen. Virol. **70:**789–800.
44. **Santoro, M. G., A. Benedetto, S. Zaniratti, E. Garaci, and B. M. Jaffe.** 1983. The relationship between prostaglandins and virus replication: endogenous prostaglandin synthesis during infection and the effect of exogenous PGA on virus production in different cell lines and in persistently infected cells. Prostaglandins **25:**353–364.
45. **Santoro, M. G., M. Fukushima, A. Benedetto, and C. Amici.** 1987. PGJ2, a new antiviral prostaglandin: inhibition of Sendai virus replication and alteration of virus protein synthesis. J. Gen. Virol. **68:**1153–1158.
46. **Santoro, M. G., B. M. Jaffe, and M. Esteban.** 1983. Prostaglandin A inhibits the replication of vesicular stomatitis virus: effect on virus glycoprotein. J. Gen. Virol. **64:**2797–2801.
47. **Saydam, O., B. Vogt, M. Ackermann, and M. Schwyzer.** 2002. Search for physical interaction between BICP0 of bovine herpesvirus-1 and p53 tumor suppressor protein. Vet. Microbiol. **86:**95–102.
48. **Schwyzer, M., U. V. Wirth, B. Vogt, and C. Fraefel.** 1994. BICP22 of bovine herpesvirus 1 is encoded by a spliced 1.7-kb RNA which exhibits immediate-early and late transcription kinetics. J. Gen. Virol. **75:**1703–1711.
49. **Serhan, C. N., J. Z. Haeggstrom, and C. C. Leslie.** 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J. **10:**1147–1158.
50. **Urade, Y., and O. Hayaishi.** 2000. Biochemical, structural, genetic, physiological, and pathophysiological features of lipocalin-type prostaglandin D synthase. Biochim. Biophys. Acta **1482:**259–271.
51. **Urade, Y., and O. Hayaishi.** 1999. Prostaglandin D_2 and sleep regulation. Biochim. Biophys. Acta **1436:**606–615.
52. **Urade, Y., and O. Hayaishi.** 2000. Prostaglandin D synthase: structure and function. Vitam. Horm. **58:**89–120.
53. **Urade, Y., K. Watanabe, and O. Hayaishi.** 1995. Prostaglandin D, E, and F synthases. J. Lipid Mediat. Cell Signal **12:**257–273.
54. **Versteeg, H. H., P. M. van Bergen en Henegouwen, S. J. van Deventer, and M. P. Peppelenbosch.** 1999. Cyclooxygenase-dependent signalling: molecular events and consequences. FEBS Lett. **445:**1–5.
55. **Wirth, U. V., C. Fraefel, B. Vogt, C. Vlcek, V. Paces, and M. Schwyzer.** 1992. Immediate-early RNA 2.9 and early RNA 2.6 of bovine herpesvirus 1 are 3′ coterminal and encode a putative zinc finger transactivator protein. J. Virol. **66:**2763–2772.
56. **Ye, Q., and H. J. Worman.** 1995. Protein-protein interactions between human nuclear lamins expressed in yeast. Exp. Cell Res. **219:**292–298.
57. **Zhang, Y., and C. Jones.** 2001. The bovine herpesvirus 1 immediate-early protein (bICP0) associates with histone deacetylase 1 to activate transcription. J. Virol. **75:**9571–9578.